

Claims

1. An assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase.
2. A homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants.
3. A homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer.
4. A homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates, e.g. by immobilizing, binding or converting pyruvates.
5. A homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and



- 33 -

15. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is pyruvate carboxylase.

16. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is pyruvate oxidase.

17. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is lactate dehydrogenase.

18. An assay as claimed in claim 6 wherein the sample is filtered with a 30 kD exclusion filter.

19. An assay as claimed in any one of claims 1 to 18 wherein said homocysteine converting enzyme is HDS and wherein a  $\text{NAD}^+/\text{NADH}$  cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

20. A kit for a homocysteine assay, said kit comprising:

homocysteine desulphurase, preferably (i) in lyophilized form, the lyophilisate being substantially free of thiol-containing cryo/ lyoprotectants or (ii) in aqueous liquid form further containing a dithiol reducing agent (e.g. DTT, DTE or TCEP) and a proteinaceous or non-proteinaceous stabilizer;

a homocyst(e)ine standard, preferably a plurality of standards containing HCy or homocystine at a plurality of known concentrations;

a reducing agent, e.g. dithiothreitol, dithioerythiol, TCEP or methyl iodide;

an agent which binds, oxidizes or depotentiates the reducing agent, e.g. an organic disulphide or a dithiol

- 34 -

binding agent, preferably a maleimide;

optionally one or more further reagents capable of converting the homocysteine conversion product of homocysteine desulphurase into a detectable analyte;

preferably a pyruvate deactivating agent, e.g. hydrazine, acetoacetate decarboxylase, pyruvate carboxylase, hydrogen peroxide or pyruvate dehydrogenase;

optionally a filter for removing pyruvate, i.e. a molecular sieve; and

optionally a filter capable of removing red blood cells from blood.